International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume 6 Issue 7, November-December 2022 Available Online: www.ijtsrd.com e-ISSN: 2456 – 6470

High Performance Liquid Chromatographic Technique and Validation for Determination of Favipiravir in Bulk & Tablet Formulation

Ms. Pritee R. Atkar, Dr. Mirza Shaihed Baig

Y. B. Chavan College of Pharmacy, Aurangabad, Maharashtra, India

ABSTRACT

Favipiravir is an antiviral that is active against many RNA viruses, & also used in COVID-19. A simple, economical, accurate, and precise HPLC method with UV detection was developed to quantify Favipiravir. It was resolved on the C18 column using the mobile phase blend of methanol: acetonitrile: ortho phosphoric acid in an isocratic mode, flow rate of 1.0 mL/min with a proportion of 40:30:30%,v/v/v. The detector wavelength was set at 322 nm. Reverse phase HPLC method, using UV detector is developed for the estimation of Favipiravir API. Used Liquid chromatographic system from Shimadzu comprising of Auto sampler, quaternary gradient pump for constant flow and constant pressure delivery and UV-Visible detector connected to software Lab solutions for controlling the instrumentation as well as processing the generated data. A mixture of Methanol: Acetonitrile: Ortho phosphoric acid in the ratio 40:30:30 v/v/v was used in RP-HPLC as diluent and as a mobile phase.

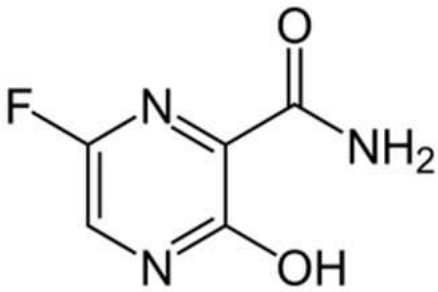
KEYWORDS: *Favipiravir, Valadation, Accuracy, Precision, LOD,*

How to cite this paper: Ms. Pritee R. Atkar | Dr. Mirza Shaihed Baig "High Performance Liquid Chromatographic Technique and Validation for Determination of Favipiravir in Bulk & Tablet Formulation" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-6 | Issue-7, December 2022, pp.719-725, URL: www.ijtsrd.com/papers/ijtsrd52424.pdf

IJTSRD52424

INTRODUCTION

FVP is an antiviral medication created by Toyama Chemical for the treatment of influenza. It prevents viral replication by specifically inhibiting the RNA polymerase of RNA viruses.[1]It has molecular formula C5H4FN3O2 and a molecular weight of 157.104 g/mol.[4] No chromatographic method with fluorimetric detection has been developed for quantitation of FAV neither in pure form nor in pharmaceutical preparation.[3] Favipiravir (6-fluoro-3-hydroxypyrazine-2-carboxamide) is an analog of pyrazine It selectively inhibits the RNA polymerase of RNA viruses, thus preventing viral reproduction. It displays antiviral activity against alpha-, filo-, bunya-, arena-, flavi-, and noroviruses, as well as being active against the influenza virus.[2] FVP is not officially available in any pharmacopoeia and there is still a need for validated HPLC methods to determine FVP in pharmaceutical formulations.[2]



Chemical structure of Favipiravir

Materials and Methods

Method development- A reverse phase high performance liquid chromatography (RPHPLC) method was developed for the estimation of Favipiravir API. The separation was achieved on Shimadzu C18 analytical column (250 mm × 4.6 mm i.d., 5 µm) using Methanol: Acetonitrile: 0.1% Ortho phosphoric acid in the ratio 40:30:30 v/v/v as mobile phase and at a flow rate of 1.0 ml/min. Detection was

carried out using a UV detector set at 322 nm. The total chromatographic analysis time per sample was about 6.0 min with Favipiravir eluting at retention time of about 3.409 min.

Chromatographic conditions set as below

Table No. 1

Mobile Phase	Methanol: Acetonitrile: 0.1% Orthophosphoric acid (40:30:30 v/v/v)
Column	C18, 4.6 mm x 2.5 cm, 5 µm (Make- Shimadzu)
Flow rate	1.0 ml/min
Injection volume	10 µl
Wavelength	322 nm
Oven temperature	Ambient
Run time	6 min

0.1% orthophosphoric acid preparation- 1 ml of orthophosphoric acid was mixed with water and diluted to 1000 ml with HPLC grade water. Sonicated for 5 minutes and filtered through 0.45 µ filter paper.

Mobile phase- Mixed 400 ml of Methanol: 300 ml of Acetonitrile and 300 ml of 0.1% Orthophosphoric acid. Filtered through 0.45 µ filter paper. Sonicated for 5 minutes.

Solution preparation-Weighed 10 mg of Favipiravir and dissolved it in 10 ml of mobile phase -Stock solution (1000 ppm) 2 ml of Stock solution diluted to 100 ml with mobile phase. (20 ppm)

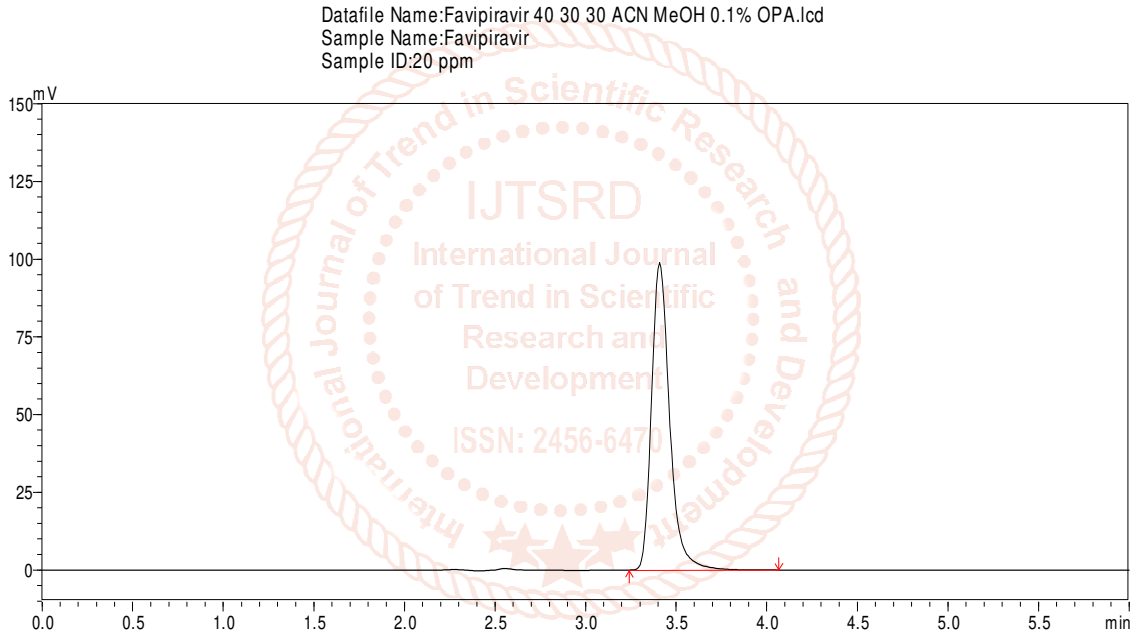


Figure1

Table No.2

Name	Ret. Time	Area	Area%	Asymmetry	Theoretical Plates
Favipiravir	3.409	692167	100.000	1.304	5233

This method found suitable w.r.t. peak shape, RT and system suitability parameters. So it is finalised for method validation. System suitability

Table No.-3

Name	Area	Retention Time	Theoretical plates	Asymmetry
Favipiravir	692167	3.409	5233	1.304
Limit	**-------**	**----**	**NLT 1500**	**NMT 2.0**

Details of Chemicals used-

Table No.-4

Chemicals/ Solvents used	Grade	Make
Acetonitrile	HPLC Gradient	Finar Ltd.
Water	HPLC	Finar Ltd.
Ortho Phosphoric acid	SQ	Qualigens
Methanol	HPLC Grade	Finar Ltd.

Method validation: The method of analysis was validated for the parameters like Accuracy, Linearity, Precision, Recovery, Robustness, LOD and LOQ.

Accuracy: Accuracy is a measure of the closeness of the experimental value to the actual amount of the substance in the matrix. The accuracy of the method was determined by calculating percentage recovery of Favipiravir For the drug, recovery study was carried out by applying the method to known label claim tablet solution known amount of Favipiravir corresponding to 50%, 100% and 150% of drug sample had been added (standard addition method). The results obtained were as depicted in **table12**.

Linearity: The linearity of a test procedure is its ability (within a given range) to obtain test results proportional to the concentration (amount) of analyte in the sample. Linearity was studied by diluting the volume of standard stock solution (1000 µg/ml) equivalent to 1.0, 2.0, 2.5, 3.0, 3.5 and 4.0ml to 100ml with same composition of mobile phase to obtain 10, 20, 25, 30, 35 and 40µg/ml working solutions respectively. All of these solutions were injected to aforesaid chromatographic conditions to quantitatively estimate the area and equivalent concentration. The results were shown in table 5.

Precision: Precision measures of how close individual measurements are to each other. All of these solutions were injected in predetermined chromatographic conditions and observed for various parameters and found within limit. Mean area was subjected to statistical analysis to determine percent RSD and found within limit as per ICH guideline Q2R1. The results were depicted in table 14,15,16,17.

Recovery: It is calculated by following formula-

Recovery = [Amount found-amount sample]/amount standard spiked*100

The obtained results were depicted in **table 13.**

Robustness: The robustness of an analytical method is a assessment of its capability to stay unaffected by small, but purposeful variations in method parameters and provides an indication of its consistency throughout customary usage. There are not much variation in the results obtained w.r.t. area after the deliberate changes done in wavelength and flow rate in case of Favipiravir. Which demonstrates that the developed method is robust and accurate. The resuts were shown in **table 6,7.**

Limit of Detection [LOD] & Limit of Quantification[LOQ]: Calculated based on the standard deviation of the response (Sy) of the curve and the slope of the calibration curve (S) at levels. Limit of detection(Lod) = 3.3*standard deviation/ slope,

$LOD = 3.3(Sy/S) = \frac{3.3 \times 8117.4}{34230} = 0.78$ µg/ml

Limit of quantification (Loq) = 10* standard deviation/slope,

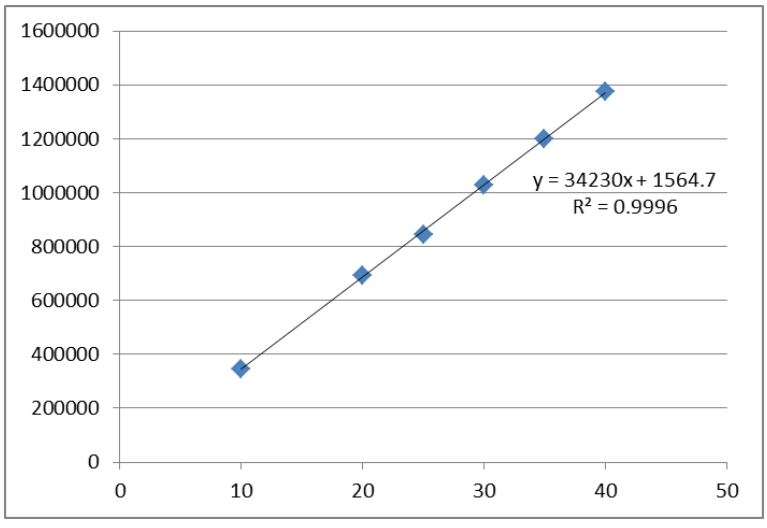
$LOQ = 10(Sy/S) = \frac{10 \times 8117.4}{34230} = 2.37$ µg/ml

Result and Discussion

Calibration curve-

Table no.-5

Conc. in ppm	Area
10	346707
20	692521
25	843522
30	1027641
35	1200122
40	1375655



Robustness

Two parameters changed-

1. Change in wavelength i.e. ± 2 nm

	WAVELENGTH (WL)	
WL	320 nm	324 nm
Area	747131	751351
% Relative Standard Deviation for Area obtained by setting 2 WL		0.398%

Table No.-6

2. Change in Flow i.e. ± 0.1 ml/min

1. 0.9 ml/min 2. 1.10 ml/min.

	Flow Rate	
Flow	0.9 ml/min	1.10 ml/min
Area	767161	761648
% Relative Standard Deviation for Area obtained by setting 2 flow rates		0.51%

Table No.-7

Accuracy

Area obtained for Favipiravir tablets

Inj. No.	Area
1	734558
2	733037
AVG	**733797**

Table No.-8

Formula for Assay % Assay = Avg. area of sample solution x conc. of std x purity x 100
Area of standard solution x conc. of sample

% Assay = 733797 x 20 x 100 x 100 = 98.7%
743066 x 20 x 100

Assay of Tablets – Inj. 1

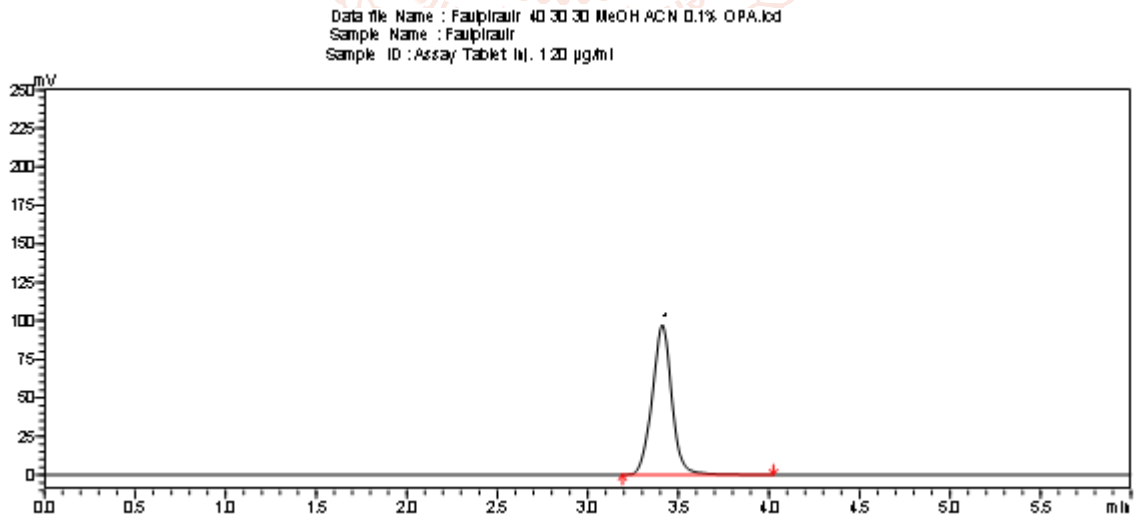
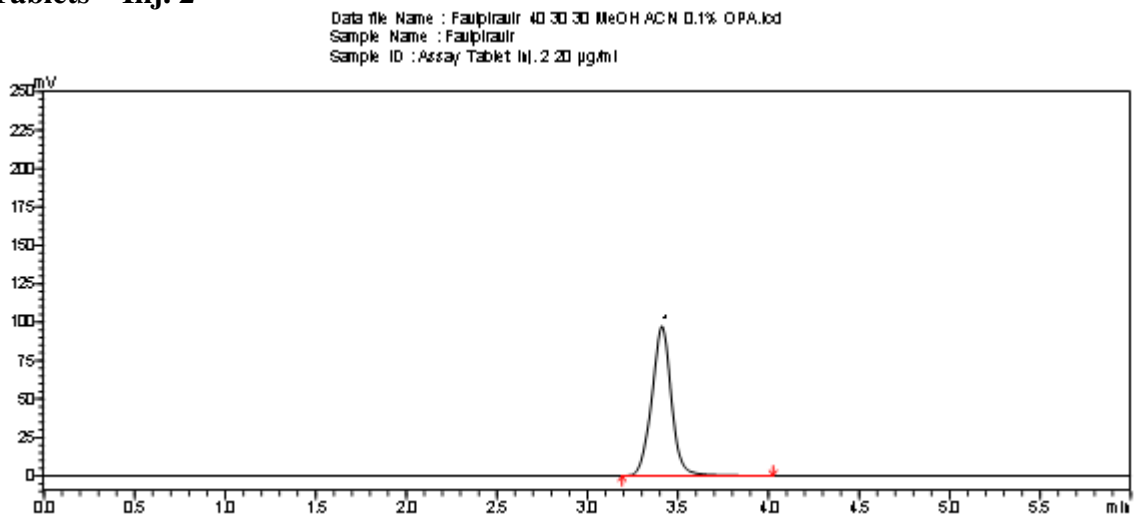


Table No.-9

Name	Ret. Time	Area	Area%	Asymmetry	Theoretical Plates
Favipiravir	3.410	734558	100.000	1.005	5380

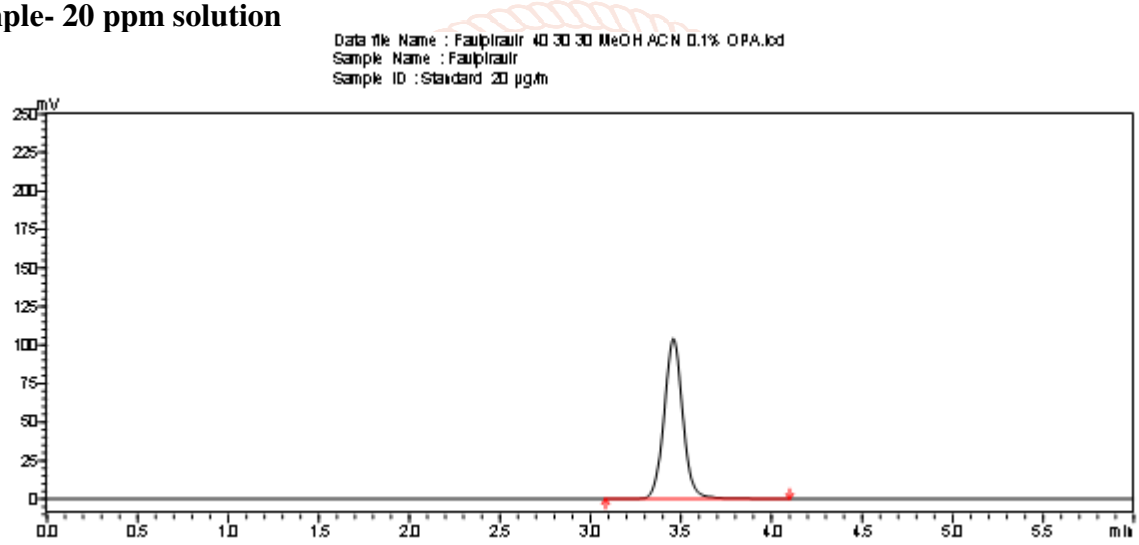
Assay of Tablets – Inj. 2



Name	Ret. Time	Area	Area%	Asymmetry	Theoretical Plates
Favipiravir	3.410	733037	100.000	1.005	5308

Table No.-10

Drug sample- 20 ppm solution



Name	Ret. Time	Area	Area%	Asymmetry	Theoretical Plates
Favipiravir	3.411	743066	100.000	1.047	5156

Table No.-11

Recovery

Level	Favipiravir Stock solution taken	Tablet Stock Solution taken	Diluted with Mobile phase	Favipiravir (in ppm)
I (50%)	1.0 ml	2.0 ml	100 ml	30
II (100%)	2.0 ml	2.0 ml	100 ml	40
III (150%)	3.0 ml	2.0 ml	100 ml	50

Table No.-12

Result Table – % Recovery of Favipiravir

Theoretical Conc. (t)	area obtained (m)	Slope (y)	Observed conc. in µg/ml (x)	% recovery
30 µg/ml	1047126	34230	30.6	102.0%
40 µg/ml	1405552	34230	41.0	102.6%
50 µg/ml	1734573	34230	50.7	101.3%

Table No.-13

PRECISION- INTRA DAY

Table No.-14

Morning 11.30 AM			
Conc.	**18 PPM**	**28 PPM**	**38 PPM**
Area	666814	996612	1363077

Afternoon 2.30 PM			
Conc.	**18 PPM**	**28 PPM**	**38 PPM**
Area	666545	997349	1363882

Evening 5.30 PM			
Conc.	**18 PPM**	**28 PPM**	**38 PPM**
Area	667160	996980	1364684

%RSD of Area calculations-

Table No.-15

Level	Conc.	Morning	Afternoon	Evening	Mean Area	%RSD
I	18 ppm	666814	666545	667160	666839	0.046%
II	28 ppm	996612	997349	996980	996980	0.037%
III	38 ppm	1363077	1363882	1364684	1363881	0.059%

%RSD of RT calculations-

Level	Conc.	Morning	Afternoon	Evening	Mean RT	%RSD
I	18 ppm	3.405	3.406	3.407	3.406	0.029%
II	28 ppm	3.406	3.408	3.407	3.407	0.029%
III	38 ppm	3.410	3.410	3.411	3.410	0.017%

PRECISION- INTER DAY

Table No.-16

DAY 1			
Conc.	**18 PPM**	**28 PPM**	**38 PPM**
Area	666814	996612	1363077

DAY 2			
Conc.	**18 PPM**	**28 PPM**	**38 PPM**
Area	666435	997094	1363860

DAY 3			
Conc.	**18 PPM**	**28 PPM**	**38 PPM**
Area	666288	998032	1364694

%RSD of Area calculations-

Table No.-17

Level	Conc.	Day 1	Day 2	Day 3	Mean Area	%RSD
I	18 ppm	666814	666435	666288	666512	0.041%
II	28 ppm	996612	997094	998032	997246	0.072%
III	38 ppm	1363077	1363860	1364694	1363877	0.059%

%RSD of RT calculations-

Level	Conc.	Day 1	Day 2	Day 3	Mean RT	%RSD
I	18 ppm	3.405	3.407	3.407	3.406	0.034%
II	28 ppm	3.406	3.406	3.410	3.407	0.068%
III	38 ppm	3.410	3.411	3.409	3.410	0.029%

References

[1] Vishal Bansi Ghune, Amit Suryakant Tapkir*, "Method development and validation of Favipiravir by RP HPLC"2022

[2] IBRAHIM BULDUK . "HPLC-UV method for quantification of favipiravir in pharmaceutical formulations" August 31, 2020

[3] Safa M. Megahed1*, Ahmed A. Habib1, Sherin F. Hammad1, Amira H.Kamal1"Chemometric approach based on factorial and Box-Behnken designs for determination of anti-coronavirus drug; favipiravir in bulk and spiked human plasma by green HPLC method"2019

[4] P. Ravisankar*, B. Divya, A. Bhavani Sailu, K. Neelima, A. Viswanath and P. Srinivasa Babu, "RP-HPLC Method for Determination of Favipiravir (RdRp of RNA Viruses) in Pharmaceutical Dosage Form"2022

[5] Duygu Taskin, "Development and Validation of a Rapid HPLC-DAD Method for Determination of Favipiravir in Pharmaceutical Formulation"2021

[6] Yousuke furuta,*1,† Takashi komeno*2 and Takaaki nakamura, "Favipiravir (T-705), a broad spectrum inhibitor of viral RNA polymerase"2017

[7] Prof. Ramarao Nadendla1* and Dr. Abhinandana Patchala**, "**A Validated high Performance Liquid Chromatographic Method for the Quantification of Favipiravir by PDA Detector"2021

[8] Hindawi Publishing Corporation Journal of Analytical Methods in Chemistry Volume 2013, **"**Development of an HPLC-UV Method for the Analysis of Drugs Used for Combined Hypertension Therapy in Pharmaceutical Preparations and Human Plasma"2013

[9] Sonu A. Varma and 1Priyanka Yadav**,** "stability indicating hplc method development and validation for estimation of favipiravir in pharmaceutical dosage form"2021

[10] Shyamala*, Dongamanti Ashok Osmania University, Hyderabad, Telangana, India., "Development of forced degradation studies of favipiravir by RP-HPLC"2021

[11] Patil Aishwarya Balu1* and Mahaparale Sonali Paresh, "stability indicating rp hplc method development for estimation of favipiravir in bulk and pharmaceutical dosage form"2021

[12] Pallavi V. Duse and Kamalkishor G. Baheti, " Bioanalytical Method Development and Validation for the Determination of Favipiravir in Spiked Human Plasma by using RP-HPLC"2021

[13] Özge Göktu˘g *, Ecem Alta¸s, Gönül Kayar and Mine Gökalp, "The Development and the Validation of a Novel Dissolution Method of Favipiravir Film-Coated Tablet"2022

[14] Application News No. L570, "Quantitative Analysis of Favipiravir Spiked in Plasma Using by HPL"

[15] Srinivas Lingbathula Neelu Jain, "Stability indicative and cost effective analytical method development and validation of Favipiravir and Peramivir in bulk and Pharmaceutical dosage form by using RP-HPLC".